

STUDIES ON
RENAL HYPERLIPEMIA

BY

ALVAR SVANBORG

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FROM

THE LABORATORY OF CLINI-
CAL CHEMISTRY, KAROLINSKA
SJUKHUSET
HEAD: LABORATOR B. SWEDIN

STUDIES ON RENAL HYPERLIPEMIA

BY
ALVAR SVANBORG

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PREFACE

Laborator Bertil Swedin and Docent Börje Olhagen have placed their great proficiency at my disposal in a manner which has been invaluable to me. They have followed my work with constant interest and facilitated its accomplishment with counsel and stimulation.

Professor Nanna Svartz has given me valuable advice and helped in every way during the course of this investigation.

I wish to express my sincere gratitude for their kindness and interest in my work, and gratefully to acknowledge the grant received from the Eightieth Birthday Fund of King Gustaf V which made this work possible.

Alvar Svanborg

INTRODUCTION

It has been known for more than 100 years that certain renal diseases are followed by a pathologic rise in the lipid content of the blood (hyperlipemia).

It has been shown by investigations carried out during the last three decades that a hyperlipemia occurs in all mammals investigated after nephrectomy or bilateral ligation of the ureters.

The causes of this hyperlipemia have not been determined. This investigation is an attempt to elucidate the cause of the hyperlipemia which occurs after bilateral ligation of the ureters in experimental animals.

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Chapter I

Review of the Literature

It has been known for more than 100 years that an increase in blood lipids follows certain renal diseases in man. In 1827, Bright (1.) stated that lactescence in the serum was a finding often observable in the renal diseases which were later collocated by posterity under the denomination Bright's disease. Bright's pupil, Christison, was the first, 1839 (2.), to point out in writing that this lactescence was dependent upon the increase of some substance, not unlike tissue fat, in the serum. Chemical analyses which confirmed Christison's supposition were first made in the beginning of the twentieth century. In 1901 (3.), Boenninger found high total lipid content in the blood of a patient with Bright's disease. In 1903 (4.), and 1905 (5.), Erben found a pathologic rise of the total lipid and lecithin content in the blood in a similar patient material. Chauffard and Laroche, 1911 (6.), reported an increase in cholesterol and Greenwald an increase in phospholipids in 1915 (7.). Bloor showed an increase also in free fatty acids and neutral fat in 1917 (8.). In 1921, Hahn and Wolff (9.) showed that the increase in cholesterol affected both free cholesterol and cholesterol ester. Gainsborough worked with a gravimetric digitonin method for the determination of cholesterol and confirmed Hahn's findings, 1929 (10.).

After Müller, 1905 (11.), formed the conception of nephrosis, Volhard and Fahr, 1914 (12.), developed their classic division of Bright's disease into nephritis, nephrosis and arteriosclerotic renal diseases. In 1916, Munk (13.) proposed the denomination lipid nephrosis.

Numerous investigators have attempted to answer the question as to which of these diseases is accompanied by a hyperlipemia. It was clarified, especially through A. A. Epstein's investigations in 1917 (14.), that hyperlipemia (hypercholesterolemia) was a common symptom in patients with lipid nephrosis. In 1921 (15.), Beumer found the same symptom in patients with amyloid nephrosis. As previously mentioned, a hyperlipemia has been found in connection with nephritis also, in the acute as well as in the chronic phase.

That the hyperlipemia occurring in cases of nephritis and nephrosis is not comprised of cholesterol alone, but includes also neutral fat, free fatty acids and phospholipids has been confirmed by many investigators; inter alia, Page, Kirk and van Slyke, 1936 (16.), Page and Farr, 1936 (17.), and Peters and Man, 1943

(18.). In the latter work, the writers point out also that the neutral fat is often more increased than the other lipid components. This is opposite to the hyperlipemia associated with hypothyrosis in which the neutral fat is often proportionally less increased than the other lipid components.

Reviews of the especially abundant literature in this field have been published by Bloor, 1917 (8.), Leiter, 1931 (19.), and Page, Kirk and van Slyke, 1936 (16.).

Hyperlipemia in experimentally induced nephrotoxic nephritis has been described by Farr, Smadel and Holden in 1937, 1939 and 1942 (20.), as well as by Cavelti and Cavelti, 1945 (21.). Their findings have been subsequently confirmed by other investigators.

The cause of the hyperlipemia in renal diseases has been discussed by several writers. It can not be secondary to a deficiency in excretory capacity as normal urine is practically free from lipids. For a review of the literature in this field, refer to Peters and van Slyke, 1946 (22.).

Epstein and Rothschild were of the opinion, 1916 (23.), that the hyperlipemia accompanying nephrosis was an instance of non-utilization of lipids derived largely from the food but partly from the break-down of tissues during malnutrition. Bloor, 1917 (8.), considered the cause of the hyperlipemia of nephritis and nephrosis to be a general metabolic disturbance brought about by a lowered alkaline reserve in blood and tissue.

In 1891 (24.), Csáthy pointed out that a decrease in plasma proteins occurred in certain forms of Bright's disease. Boggs and Morris, 1909 (25.), showed that a hyperlipemia appeared in rabbits with severe hemorrhagic anemia. Fishberg and Fishberg, 1927 (26.), believed they could prove that this anemia-hyperlipemia occurred concurrently with a decrease in serum protein. In 1929 (27.), E. H. Fishberg questioned whether the hyperlipemia in nephrosis might not be a compensatory process acting to raise the colloid osmotic pressure in the plasma in instances of low plasma protein concentrations.

These findings were collocated by Epstein in 1928 (28.) with the earlier observations that the basal metabolism was often low in patients with nephrosis. His final theory was that hypoproteinemia and hyperlipemia were associated with hypothyrosis. Many writers have opposed this conception which in its day awoke great interest. Lichtenstein and E. Z. Epstein, 1931 (29.), Bing and Starup, 1935 (30.), Page and Farr, 1936 (17.), and Peters and Man, 1943 (18.), report cases in which no definite connection between protein and lipids in serum could be shown. Peters and Man, as mentioned before, also showed a qualitative difference between the hyperlipemia of nephrosis-nephritis and that of hypothyrosis. Page and Farr (17.) obtained no effect on the hyperlipemia in nephrosis and nephritis following the administration of large doses of thyroidea for long periods. Fishberg's conception of the hyperlipemia in hemorrhagic anemia as a colloid osmotic compensation for hypoproteinemia has been strongly opposed by Popják and McCarthy, 1943 (31.) and 1946 (32.). They showed that the lipids in normal

and hyperlipemic serum do not exercise an osmotic pressure. In 1943 (18.), Peters and Man, *inter alia*, pointed out that the concentrations of both proteins and lipids decrease in serum during malnutrition, a condition which also contraindicates the existence of an inverse relation between these components.

Whether any relation exists between edema and hyperlipemia has been discussed to some extent in the aforementioned investigations, in close connection with the question of the relation between hypoproteinemia and hyperlipemia. The hyperlipemia in the majority of cases is of such severity that it can not be explained by an increase in serum concentration due to dehydration. The discussion has been particularly concerned with the cases of nephritis in which extensive edema occurs without hypoproteinemia. A review of the debate on this question has been presented by Peters and van Slyke, 1946 (22.). It may be said in summarizing, that it has not been possible to show a constant relation between peripheral edema and milder hyperlipemia, but that the most pronounced hyperlipemias are to be found reported in the patients with massive edema.

Attempts to correlate hyperlipemia with other symptoms, *e. g.*, albuminuria or rise in nonprotein nitrogen, in cases of nephritis have not been successful either. Refer to Peters and van Slyke, 1946 (22.), for a review of the literature on this subject.

The question as to whether the hyperlipemia accompanying affections of the kidney depends upon an inability of the organs to break down the lipids or upon a disturbed mechanism for the transfer of lipids from the blood to the tissue depots has been studied with varying results by a number of investigators. In the majority of cases, however, the cholesterol concentrations have been studied after the administration of fat-rich or fat-deficient diet. The results must be regarded against the background of the fact that an increase in the fat content of the diet is not followed by any large changes in the cholesterol concentration in serum in healthy persons either, a condition reported, *inter alia*, by E. B. Man and Gildea, 1932 (33.).

Hiller, Linder, Lundsgaard and van Slyke, 1924 (34.), reported a greater rise in the lecithin and total fatty acid concentration in plasma three and five hours after a fat-rich meal in patients with nephritis-nephrosis hyperlipemia than in normal persons or in nephritic patients without hyperlipemia. No definite difference in oxygen assimilation in healthy persons and patients with the hyperlipemia of nephritis or nephrosis could be shown.

In literature reviewing the field, it is said that contradictory results are reported by Page and Farr, 1936 (17.), and Odinson and Guschtschina, 1935 (35.), *inter alia*. Such is not the case, however. Page and Farr studied the blood lipids in cases of nephrosis and nephritis after longer periods of respective fat-rich and fat-deficient diet. It should be noted, however, that the analyses were made with blood taken in a postabsorptive stage; *i. e.*, in the morning after a period of fasting. They found a slight drop in the total lipid content after administration of fat-

defficient diet, but otherwise no effect. Odinow and Guschtschina found no increase in lipids in patients with nephritis three, six and nine hours after meals. A more detailed study of the publication reveals, however, that none of the patients with nephritis had hyperlipemia when the investigation was started.

In conclusion, this review of the literature should mention the investigations in which *the blood lipids were studied after nephrectomy, ligation of the ureters or ligation of renal vessels*. Nekludow, 1925 (36.), found a rapidly progressing hyperlipemia after nephrectomy or bilateral ligation of the ureters in cats. Heymann, 1942 (37.), reported the same effect in dogs. Winkler, Durlacher, Hoff and Man, 1943 (38.), investigated this condition in dogs and monkeys. They stated also that a similar hyperlipemia occurred after bilateral ligation of the ureters. A more thorough discussion of these investigations is presented in chapter IV.

Derow, Schlesinger and Savitz, 1939 (39.), and A. M. Fishberg, 1944 (40.), described hyperlipemia, albuminuria and hypoproteinemia in patients with thrombosis of the renal vein. Johnson, Bauer, Hirsch and Carbonaro, 1951 (41.), carried out experiments, based on the foregoing, with ligation of the renal artery or vein on one side during a period of 12 to 20 minutes. They subsequently obtained a marked rise in the serum lipids, especially the neutral fat, with a maximum after three to four days and a return to normal values after one to two weeks.

According to Miyazaki, 1934 (42.), and Shu Man Kyo, 1937 (43.), the hyperlipemia occurring in rabbits after bilateral nephrectomy was inhibited if renal venous blood from healthy rabbits was administered. The kidney was said to secrete a hormone which affected the lipid metabolism.

Jiménez Diaz and Castro Mendoza, 1948 (44.), were of the opinion they could show that glycerol extract from healthy kidney had a similar hyperlipemia-counteractive effect. More detailed discussion of these investigations is presented in chapter V.

Wachstein, 1946 (86.), was of the opinion he could show by a histochemical method a decrease in lipase and alkaline phosphomono-esterase activity in the "ureter-ligated" kidney in rats 10 to 12 days after unilateral ligation of the ureter. His interpretation of the results was such that "it seems conceivable that enzymes concerned with fat metabolism in the kidney may be significant for the regulation of lipid metabolism of the whole organism". The method used in that investigation and the possibilities of drawing conclusions therefrom will be discussed in greater detail in chapter III.

Chapter II

Summary of the Review of the Literature

Hyperlipemia is a symptom in cases of nephrosis and certain forms of nephritis. This hyperlipemia is dependent upon an increase in neutral fat, cholesterol, free fatty acids and phospholipids.

Similar hyperlipemia is described in cases of experimental nephrotoxic nephritis.

The cause of these hyperlipemias does not seem to have been explained previously.

A similar hyperlipemia occurs after bilateral nephrectomy or ligation of the ureters or after ligation of renal vessels (renal vein thrombosis). This hyperlipemia, according to certain writers, is said to be counteracted by the administration after nephrectomy of venous blood or glycerol extract of healthy kidney.

The Problems to be Investigated

The aim of this investigation is to ascertain the cause of the hyperlipemia which occurs after bilateral ligation of the ureters or after nephrectomy. This hyperlipemia has been found in rats, cats, dogs and monkeys.

1. Does a similar hyperlipemia occur in rabbits also following bilateral ligation of the ureters?
2. Can this hyperlipemia be counteracted if renal venous blood or extract of healthy kidney is administered?
3. Otherwise, can the cause of hyperlipemia be that the kidney normally takes part in the break-down of lipids and that this function is reduced by ligation of the ureters?
 - a. Can a conception of the ability of the kidney to break down lipids be obtained by means of lipid analyses in afferent and efferent renal blood?
 - b. Can a conception of the ability of the kidney to break down lipids be obtained through a comparison of the lipase activity in kidney and liver tissue suspensions?
 - c. How do the enzyme activities behave in kidney and liver suspensions and in serum after ligation of the ureters?

Chapter III

General Methods

1. Lipid Determination Methods

The determinations were made in blood, urine and tissue suspensions.

Extraction of the lipids was carried out as follows:

From *serum*, by the Edsgaard method, 1948 (45.). In extraction from whole blood, the blood corpuscles were hemolysed with distilled water. Alcohol 25 parts, heated to just below boiling point, to blood 1 part was used for extraction.

From *urine*, by shaking with 25 parts of petroleum ether in a shaking machine for six hours.

From *tissue suspensions*, by Soxhlet extraction with petroleum ether for five hours. (Longer extraction time did not increase the yield.)

After careful evaporation of the extraction medium, the lipids were redissolved in chloroform. From several portions of this chloroform solution, determinations (in the tables = duplicate values) were made of:

Total lipids, by means of evaporation and weighing of the residue.

Cholesterol, according to the method of Theorell and Widström, 1931 (46.), with the Liebermann-Burchard color reaction.

Phospholipids, after evaporation of the chloroform, digestion of the residue with 30 percent sulphuric acid, redissolving in water, and phosphate determination according to the method of Briggs, 1922 (47.).

2. Protein Determination Method

Total protein determination in serum was carried out according to the micro-Kjeldahl method, as described by Theorell and Widström, 1931 (48.).

3. Nonprotein Nitrogen Determination Method

Nonprotein nitrogen determination was made according to the Folin and Svedberg method, 1930 (49.).

4. Enzyme Activity Determination Methods

General views on enzyme activity determinations in the internal organs.

In the determination of the normal enzyme activity in the parenchymal ab-

dominal organs, it is preferable that the method used permit the determinations to be made while the organ functions under entirely normal conditions. The determinations must therefore take place with the organ *in situ*.

Accordingly, *one method is to determine the concentration of the substrate on which the enzyme acts in the blood in the afferent and efferent vessels, and to estimate the enzyme activity from the differences in concentration.* It is necessary to take into consideration, however, the possible accumulation of substrate in the organ, or the enzyme activity of opposite effect in the organ (e. g., synthesis, if the activity of a splitting enzyme is to be determined). If the quantity of blood which passes through the organ per unit of time is large, the differences in substrate concentration will be so small in many cases that they can not be demonstrated with the available methods. In so-called *perfusion experiments*, these differences in concentration are increased by diverting a small quantity of blood from large parts of the rest of the circulatory system and passing it repeatedly through only that organ in which the enzyme activity is to be determined. However, this causes a change in the composition of the blood and the other tissues. An important objection also is that the organ is isolated from its normal hormonal regulation. Therefore, quantitative conclusions regarding the normal enzyme activity of an organ can seldom be drawn from perfusion experiments.

In *determination of enzyme activity in an excised piece of an organ*, the normal working conditions of the enzyme are no longer present. Hence, only limited conclusions may be drawn from such analyses.

Enzymes in thin tissue slices are able to exercise enzyme activity in substrate solutions. This method has its greatest importance in the study of enzyme activity fixed in cell elements. However, the results are influenced by several factors which, not considering section technique, make the method less suitable for quantitative enzyme analyses. For example, the result is affected by the proportion of the contact surface to the total quantity of tissue, by the relation between the number of whole and fragmentary cells at the surface of the slice, by the inactive connective tissue present in the slice surface, and by the diffusibility of the enzyme and substrate through cell membranes. The methods published by Gomori (50.) and Takamatsu (51.) in 1939 for the determination of the *enzyme activity in histologic slices* are based upon this method. In addition to the objections raised, such methods have further disadvantages in quantitative enzyme determinations. The enzyme must be resistant to the tissue fixing and section technique. Some of the end products from the substrate in the solution must be able to form colored compounds. These must then adhere to the surface of the slice at the sites of the enzyme activity. Finally, the degree of the enzyme activity must be objectively interpretable from the intensity of the color in the slice.

B. Swedin and I have attempted to purify acid phosphomono-esterase in sperma plasma (84.). We were successful in obtaining a fraction which proved at electrophoretic examination to contain only two protein components. One of these was

the active enzyme. If ice-cold acetone is added to this enzyme preparation, the enzyme activity is immediately destroyed. This observation is worthy of mention in this discussion inasmuch as ice-cold acetone was used in the fixing of the tissue in the methods developed by Gomori for the determination of acid and alkaline phosphomono-esterase and lipase enzyme activity in tissue.

G. Johansen and K. Linderström-Lang, 1951 (85.), have questioned whether there is not a possibility in the test that calcium phosphate will be precipitated not at the sites of enzyme activity but at places where there are preformed crystal nuclei or cell structure elements particularly favorable for adsorption. These writers questioned whether or not spontaneous precipitation of the salt visible in histologic sections at the places of enzyme activity ever occurs when Gomori's method is used.

It must therefore be considered highly doubtful that quantitative conclusions concerning the enzyme activity can ever be drawn from the available methods of histochemical analysis. The work of Wachstein (86.), mentioned in the review of the literature, concerning the lipase activity in the kidney after ligation of the ureter must therefore be considered against this background.

After the breaking down of a tissue, there are greater possibilities for contact between the tissue enzyme and the substrate.

The tissue can be broken down by autodigestion, providing that the enzyme of which the activity is to be determined is not destroyed by the proteolytic ferment or the change of milieu during the autodigestion. The enzyme is extracted from the resultant tissue homogenate. It is often impossible, however, to determine whether the changes in enzyme activity demonstrated in pathologic conditions have arisen during the autodigestion. Thus, for example, the pathologic metabolic products present in a diseased organ can be split during autodigestion, liberating enzyme-destroying substances.

In *mechanical breaking down of tissues*, the homogenizer constructed by Potter and Elvehjem, 1936 (52.), was often used earlier. The principle of this method is the grinding of the tissue specimen by a grooved, ground, glass plunger rotating in a test tube. Only a coarse breaking down of the tissue is obtained by this means, however. Principally whole cells and groups of cells are found in a microscopic inspection. Consequently, extraction of enzymes from these cells and groups of cells can not be complete.

In order to obtain a direct contact between the enzyme of the cells and the substrate in question, it is generally preferable that the cell membranes also be broken down. In collaboration with Mr. H. Broström, engineer, I constructed a homogenizer according to the principles embodied in the Warring Blender which fulfills this requirement. Homogenizers have previously been built on similar principles by Alford and Palmes, 1944 (53.), and Folley and Watson, 1948 (54.).

Homogenizer

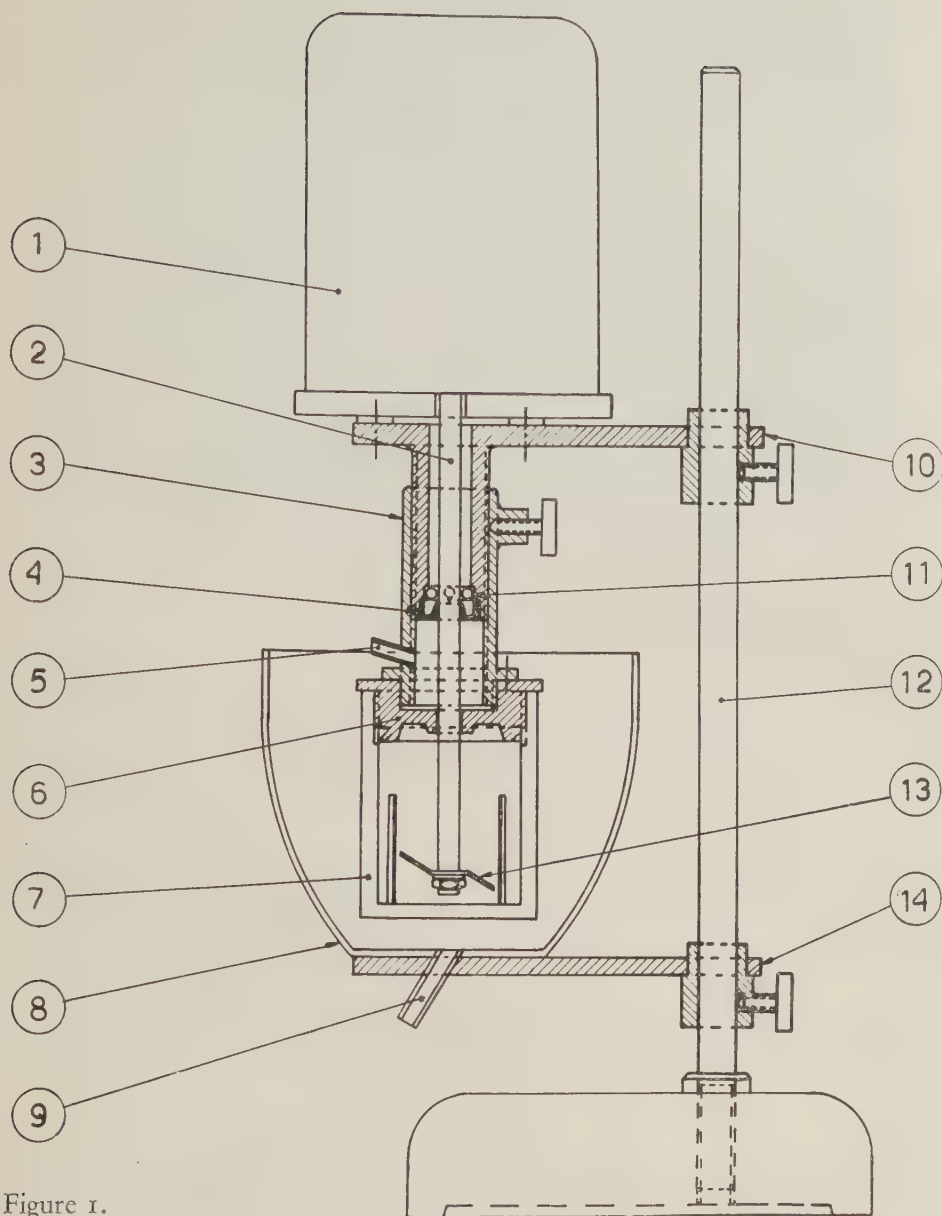


Figure 1.

1. Electric motor, maximum speed: 12 000 r.p.m.
2. Stainless steel spindle coupled directly to shaft of motor.
3. Holder for beaker assembly, adjustable height.
4. Partition and "moat" for collection of impurities from motor and bearings.

5. Gas intake.
6. Beaker holder with arched "beaker ceiling".
7. Plexiglas beaker with three equidistant, vertical, inside "fins" in the wall. The plexiglas beaker is screwed fast to the beaker holder.
8. Outer cooling jacket.
9. Drainage tap from cooling jacket.
10. Homogenizer holder, adjustable height.
11. Ball-bearings, double row.
12. Stand.
13. Stainless steel knives.
14. Cooling jacket holder.

This homogenizer has the following advantages:

1. Plexiglas is durable under vibration and other mechanical strains. It makes effective, air-tight screw-fastening of the beaker to the holder possible. In such manner, contact is also avoided between the enzyme and the rubber packing and other parts which can have an enzyme-destroying effect. Plexiglas (specific gravity, about 1.2) is lighter than ordinary glass (specific gravity, about 2.6). Because of its greater durability, the walls of the beaker can be thinner than with ordinary glass. These beakers will therefore be lighter, permitting greater accuracy in the weighing of small quantities of tissue. Finally, plexiglas is easy to turn on the lathe, to thread, et cetera.

In the enzyme determinations made in this investigation, no differences in activity were shown whether the tissue suspensions were kept in glass or in plexiglas. The enzyme activities were the same after 10 or 60 minutes' homogenization. The conclusion has been drawn from this that the enzymes investigated in this work are not destroyed by contact with plexiglas.

2. Homogenization takes place in a "closed" container. The excess gas pressure counteracts fluid loss by absorption along the spindle. An important advantage is that the homogenization can take place in the desired gas milieu.

The gas is introduced under rather high pressure into the beaker before it is completely screwed fast. After the air is forced out, the beaker is screwed into place.

3. Impurities such as lubricating oil from the motor have proved to inactivate the enzymes investigated in this work. With this construction, such contamination is impossible.

4. Because of the design of the "beaker ceiling", bits of tissue that are thrown up are thrust down into the solution again. If the homogenization takes place in a glass tube or similar container, the bits of tissue thrown up stick to the walls and fail to be homogenized.

5. Inasmuch as the beaker assembly is adjustable in height, beakers of varying depth may be used when necessary. The beaker assembly and knives can be removed by a twist of the wrist and exchanged if beakers of other dimensions are needed.

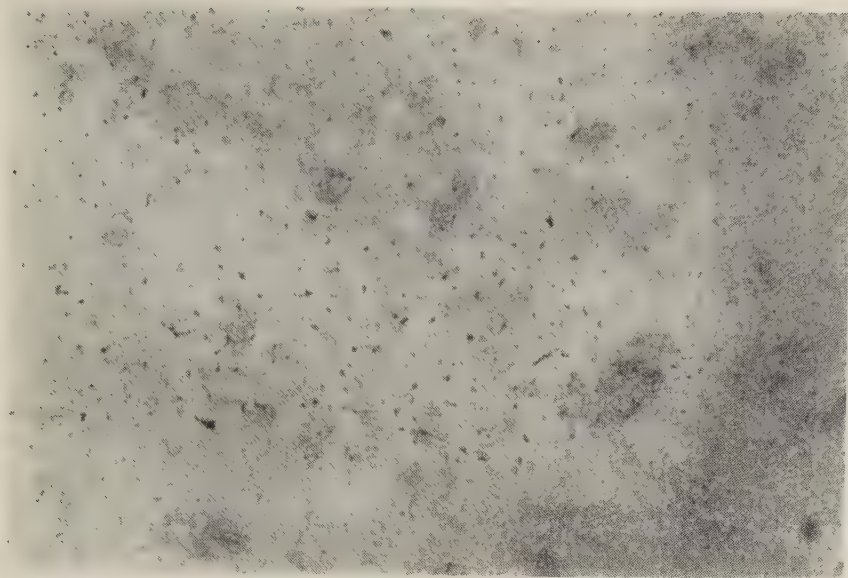


Figure 2.

Estimation of the results of homogenization with this homogenizer

1. In the homogenization of kidney or liver, such complete destruction of the cells is obtained that, on the whole, only cell and nuclear fragments with no whole parenchymal cells can be observed microscopically (see figure 2). The parenchymal cells are peeled off of coarser connective tissue fibers but the fibers remain otherwise whole and sink to the bottom of the beaker after the homogenizer is stopped.

2. The dry weight has been determined in different parts of the tissue suspension by drawing off two portions of 1 ml. each of the homogenate, using the same pipette. The mean dry weight in these portions appears in table 1. The results show that the distribution of solids in the various parts of the suspension is practically homogenous.

3. The double values obtained in the enzyme activity determinations reported in chapter VII can serve for further estimation of the dispersity.

Table 1.
Dry weight in tissue suspensions.

	Dry weight in mg. Mean value	Error of method in per- cent of mean value	Number of deter- minations
Kidney	18.0	0.75	65
Liver	21.2	0.75	46

Error in method computed according to formula $S^2 = \frac{\sum d^2}{2n}$, in which d = difference between 2 determinations and n = number of determinations.

Methods Used in Enzyme Activity Determinations

The determinations were made in blood, and in kidney and liver suspensions.

A tissue fragment weighing approximately 3 Gm. was taken consistently from the same part of the organ immediately after the animal was sacrificed for analysis of enzyme activities. Hilus connective tissue was removed from kidney tissue. The tissue to be homogenized was cut into pieces with scissors. About 0.5 Gm. of the resulting small pieces was weighed in the beaker of the homogenizer. Homogenization of the pieces of tissue was carried out with the homogenizer described earlier, in 10 ml. physiologic saline solution at 0°C to +4°C and in an atmosphere of nitrogen. The homogenization time was greater than 10 minutes.

Two homogenates were prepared from each organ. The duplicate values given in the tables refer to the enzyme activity determinations in these two homogenates.

The activity of the enzymes was the same whether the homogenization took place in air or nitrogen. Maximal dispersion of the tissue in the suspension was attained after 10 minutes' homogenization.

Determination of lipase (tributyrase) activity was carried out, on the whole, according to the method of Goldstein, Epstein and Roe, 1948 (55.).

A solution was made from 0.75 ml. tributyrin (Fisher Scientific Co., Eimer and Amed) plus 30 ml. 0.5 percent diethyl sodium solution plus 30 ml. calcium acetate solution (20 Gm. calcium acetate, chemically pure $\text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$, dissolved in distilled water and the solution made up to 1 liter) and shaken in a mechanical shaker one hour. From this solution, 20 ml. is drawn off with a pipette and placed in a 200 ml. Erlenmeyer flask. After 1 ml. enzyme solution is added, the whole is incubated at 37°C for one hour. The enzyme activity is interrupted by the addition of 100 ml. ether-alcohol (96 percent alcohol 9 parts to ether 1 part). Control experiments were carried out in the same manner, except that the enzyme solutions were not added until after the addition of the alcohol-ether. Both experimental and control suspensions were titrated to a pink color with 0.05 normal KOH, using phenolphthalein as indicator. The difference in the titration values of the experimental and control suspensions is a measure of the quantity of fatty acid liberated from tributyrin.

If the enzyme suspensions were heated slowly to 70°C for 10 minutes before incubation, no enzyme activity could be determined.

In order to decrease the errors in measurement which occur in the pipetting of suspensions, the same pipette, a 20 ml. volumetric pipette with a white tip, was used in the pipetting of substrate suspension in all determinations. A 1 ml. graduated white-tipped pipette from the same manufacturer was used for the enzyme suspensions.

Determination of the activity of alkaline phosphomono-esterase was carried out exactly according to the method of Buch and Buch, 1939 (56.).

The activity of acid phosphomono-esterase was determined by the same method. However, the veronal buffer was replaced by an 0.11 mol citrate buffer of pH 4.8. The incubation period was 30 minutes.

Phosphomono-esterase activity has been expressed in millimol liberated phenol.

If the tissue suspensions were heated to 70°C before incubation, no enzyme activity could be demonstrated subsequently.

Chapter IV

Investigations of Serum Lipid Concentrations after Ligation of the Ureters

Nekludow, 1925 (36.), discovered that a hypercholesterolemia followed nephrectomy or ligation of the ureters in cats. Heymann, 1942 (37.), reported an increase in the cholesterol and total lipids in dogs after bilateral nephrectomy. He demonstrated a transient and slighter increase in lipids following unilateral nephrectomy. The serum protein concentration was constant despite the lipid increase. In 1943, Winkler, Durlacher, Hoff and Man (38.) published an extensive investigation of hyperlipemia following ligation of the ureters or nephrectomy. Dogs and monkeys were used in the experiments. In both these species, but especially pronounced in monkeys, a rapidly progressing hyperlipemia appeared, including neutral fat as well as free fatty acids, free cholesterol, cholesterolesters and phospholipids. No decrease in the total proteins in the serum was observed. Unilateral nephrectomy or splenectomy under nembutal anesthesia did not affect the blood lipids. The dogs were starved 10 days without a resultant comparable effect on blood lipids. In 1945, Heymann et al. (57.) published a new investigation which, on the whole, confirmed the earlier results. As in 1942, however, they found a slight rise in blood lipid concentrations following unilateral nephrectomy, although similar findings were obtained following sham operations and splenectomies. In rats also, a rise in the blood cholesterol followed nephrectomy. In conclusion, the writers raised the question as to whether the kidney is not linked, in some way, with the normal lipid metabolism. Ludwig and Chanutin reported in 1938 (58.) that after administration of cholesterol-rich diet, the cholesterol concentration in the blood of rats with partial nephrectomy was higher than in that of normal animals. The writers suggested therefore that the cholesterol disturbances in renal diseases of man are directly associated with renal damage.

The following is my investigation of serum lipid concentrations following unilateral and bilateral ligation of the ureters in rabbits

Rabbits from a mixed collection of experimental animals were used. Weight and sex is given in the table. The animals received a standard diet consisting of oats, hay and turnips.

Pentothal was used as anesthetic. A 2 to 3 cm. incision was made in the abdominal midline immediately above the symphysis. Thereafter, the ureters, which are easily accessible at this site, were ligated a few centimeters proximal to their entrance to the bladder. The

Table 2.

Lipid concentration in serum after bilateral ligation of the ureters.

No. Weight in grams	Sex	Days after liga- tion	Total lipids mg./100 ml. d.v. m.v.	Cholesterol mg./100 ml. d.v. m.v.	Phospholipids mg./100 ml. d.v. m.v.	N.P.N. mg./100 ml.
UR ₃		0	510	56	3.1	
2190			560	55	3.1	37
			610	53	2.9	
♂		3	1920	102	7.2	300
			2030	102	7.9	
			2130	102	8.3	
UR ₁₅		0	275	23	1.0	
2310			290	23	1.8	37
			310	23	1.0	
♂		3	960	54	5.8	
			960	54	6.3	121
			965	54	5.3	
UR ₁₇		0	265	47	3.6	
3320			300	51	3.8	31
			335	55	3.8	
♂		3	1360	85	9.0	
			1370	86	9.4	220
			1380	87	9.0	
		4	1225	120	10.0	
			1230	122	10.4	288
			1229	124	10.4	
UR ₁₈		0	383	50	4.2	
4000			390	51	4.2	28
			388	52	4.2	
♂		3	5010	320	21.4	180
			4930	320	20.5	
			4850	320	19.5	
UR ₄		0	307	33	3.2	
2460			310	35	3.3	28
			307	36	3.4	
♂		1	770	90	5.7	
			860	90	7.0	5 ml./kg. body weight per day
			950	90	6.2	
		2	1770	106	7.9	
			1670	107	8.1	126
			1570	108	8.0	
					8.1	

	0	380	460	13	1.7	1.7	4.3	6 ml./kg. body weight per day
UR ₅								
2530		540		13	1.7			
♂	2	1270	1280	25	3.6			
		1280		25	3.6	3.6	129	
					3.7			
	4	2140	2130	114	9.4			
		2110		120	11.4	10.5	202	
					10.6			
UR ₇	0	695		50	3.9			7.5 ml./kg. body weight per day
1900		740	720	52	4.3	4.1	—	
				54	4.1			
♂	3	1500	1510	72	5.6			
		1520		76	5.9	5.8	245	
UR ₁₆	0	379		35	2.8			Injection of physiol. saline solution extract from 6 gm. healthy rabbit kidney per day
4750		433	410	35	3.0	2.9	29	
				35	2.8			
♂	2	2986	2990	109	13.9			
		2990		109	14.3	14.0	140	
					13.9			
UR ₁	0	280		33	2.6			Injection of glycerol extract from 3 gm. healthy rabbit kidney per day
2710		305	290	35	3.0	2.7	30	
				37	2.6			
♂	3	3970	4100	175	19.1			
		4230		198	19.7	19.5	—	
					19.7			
UR ₈	0	925		47	8.9			Injection of glycerol extract from 9 gm. healthy guinea pig kidney per day
1940		974	950	51	9.3	9.1(?)	34	
								Replacement transfusion
♀	2	1770	1800	138	9.7	9.9	134	
		1830		141	10.0			
UR ₁₄	0	573		—	1.7			Injection of glycerol extract from 3 gm. healthy pig kidney per day
2290		573	570		2.0	2.0	—	
					2.3			Replacement transfusion
♂	2	1650	1730	119	11.0			
		1810		127	12.0	11.4	164	
					11.3			

Volume of replacement transfusion = volume of blood withdrawn for analysis.

abdomen was closed. The time for the operation was about five minutes. The duration of the narcosis was 10 to 20 minutes. This operation can be performed almost bloodlessly.

Blood samples for lipid analysis were taken from the vessels of the ear and by means of cardiac puncture. Only serum without macroscopic hemolysis was used for analysis.

Bilateral ligation of the ureters was performed on 11 rabbits (UR = "Ureter-ligated" Rabbit). Seven of these rabbits were treated in the manner described in greater detail in chapter V. The first 24 or 48 hours after the operation, the animals were comparatively unaffected and ate more or less normally. With individual variation, they later became more and more affected. The animals were sacrificed by bloodletting after cardiac puncture, thereby achieving the advantageous severe anemia of the internal organs. The results of the lipid analyses in serum appear in table 2.

The lipid concentrations before and after *unilateral ligation* of the ureters were determined in three animals. In an additional two, lipid concentrations in serum were determined only after sacrifice (see table 3).

Table 3.
Lipids in serum after unilateral ligation of the ureter.

No. Weight in grams Sex	Days after li- ga- tion	Total lipids mg./100 ml.		Cholesterol mg./100 ml.		Phospholipids mg./100 ml.		N.P.N. mg./100 ml.
		d.v.	m.v.	d.v.	m.v.	d.v.	m.v.	
UR ₂ 2070 ♂	0	550		49		4.0		
			620		51	4.1	4.0	
		696		53		4.0		
	4	445		56		2.5		
			460		62	1.9	2.3	43
		475		67		2.5		
UR ₉ 1890 ♂	0	610		89				
			630		90			
		650		91				
	6				w.b.			
		570		98				
			640		98			40
UR ₆ 4120 ♀	0			75				
					75			
					w.b.			
	23			87				
				84				43
					86			
UR ₁₀ 2530 ♂	4	900		81		5.0		
			920		83	5.0	5.0	
		930		84		5.0		
					w.b.			
UR ₁₁ 3170 ♂	10	625		88		3.2		
			630		88	3.5	3.3	
				88		3.2		
					w.b.			

w.b. = determinations made in whole blood.

d.v. = duplicate values

m.v. = mean value

As is apparent from the tables, a rapidly progressing hyperlipemia occurs in rabbits after bilateral ligation of the ureters. Following unilateral ligation, on the other hand, no definite rise in the lipid concentrations could be demonstrated in this limited material.

The analyses after unilateral ligation show, however, that the anesthetic, the operation and the loss of blood in connection with the taking of samples for testing did not cause the hyperlipemia which followed bilateral ligation of the ureters. In addition, in six of the rabbits with bilateral ligation, the total blood volume was held constant by injection of the same amount of blood from a healthy rabbit as was withdrawn for analysis. Controls of serum protein concentration in these animals showed unchanged values during the course of the investigation. The conclusion was drawn from these conditions that the hyperlipemia occurring after bilateral nephrectomy could not be secondary to a hypoproteinemia.

Two rabbits were starved. After 12 days, a slight increase in blood lipids could be observed (see table 4). In lipid analyses made so soon after bilateral ligation of the ureters that the animal was still comparatively unaffected and ate more or less normally, hyperlipemia was found notwithstanding. This precluded the possibility that the hyperlipemia observed depended upon starvation.

Study of the kidneys with ligated ureters revealed enlarged, edematous kidneys, sometimes with capsular hemorrhages. Microscopic examination of the kidneys of the animals with bilateral ligation of the ureters showed an extensive edema and dilated tubules, especially in their distal portions. In those from the animals with unilateral ligation, particularly when the animals were sacrificed a longer time after the ligation, more severe changes and atrophy of the cells of the tubules as well as extremely dilated canals were observed. In all kidneys examined, the glomeruli appeared to be intact. Fat staining showed no pathologic renal fatty

Table 4.
Lipids in serum after starvation.

No. Weight in grams Sex	Starva- tion days	Total lipids mg./100 ml.		Cholesterol mg./100 ml.		Phospholipids mg./100 ml.		Plasma proteins Gm./100 ml.
		d.v.	m.v.	d.v.	m.v.	d.v.	m.v.	
NR ₁₂	0	370				1.4		
3160			430	14	14	1.7	1.5	8.1
♂		480				1.4		
2550	12	470				3.5		
			510	91	91	3.5	3.6	6.9
		545				3.8		
NR ₁₃	0	275				1.2		
3460			280	15	15	1.4	1.4	7.3
♂		291				1.6		
2530	12	542				3.9		
			570	89	89	4.2	4.0	6.9
		596				3.9		

NR = Normal (healthy) Rabbit

degeneration. These changes agree closely with those in earlier attempts to produce hydronephrosis, as reported by a number of writers (see, inter alia, Johnson, 1918 (59)).

Total lipid determination was made in suspensions of renal tissue from two healthy rabbits and from two with hyperlipemia following bilateral ligation of the ureters. It was also determined in renal tissue suspensions from both kidneys in two rabbits with unilateral ligation (see table 5). It is apparent from these values that no deposit of lipids was observed in renal tissue after ligation of the ureters.

Table 5.
Concentrations of lipids in normal and in "ureter-ligated" kidneys.

No.	% total lipids in dry weight	
NR ₄	14.9	Normal Kidney
NR ₆	16.8	Normal Kidney
UR ₃	11.9	3 days after bilateral ligation of ureters
UR ₅	10.4	4 days after bilateral ligation of ureters
UR ₂	8.6	Normal Kidney
	7.8	4 days after unilateral ligation of ureter
UR ₆	11.9	Normal Kidney
	11.9	23 days after unilateral ligation of ureter

Thus, in summing up, it may be said that these investigations showed that after bilateral ligation of the ureters a rapidly progressing hyperlipemia occurs in rabbits. Corresponding hyperlipemia was not obtained after unilateral ligation. Lipid concentrations were unchanged in renal tissue after ligation of the ureter.

Chapter V

Investigations as to whether a Hormone which Regulates the Lipid Metabolism is Formed by the Kidneys

Miyazaki, 1934 (42.), reported the results of investigations supposed to show that renal venous blood from healthy rabbits, in doses of 2.0 ml. per kilogram body weight, was able to lower the cholesterol content in peripheral blood of healthy rabbits and counteract hyperlipemia following nephrectomy. A similar effect was not obtained with peripheral venous or arterial blood. The maximal effect was reached after six to seven hours; the effect endured 24 hours. In his opinion, a lipid-metabolism-regulating hormone was formed in the cells of the tubules. He also reported demonstrating that this renal hormone was produced in the cells of the tubules. The effect failed to appear with renal venous blood from rabbits in which the cells of the tubules were damaged with potassium chromate. Finally, Miyazaki stated that the hormone was insoluble in water but soluble in alcohol, acetone, chloroform and ether. It was not saponified and was not destroyed by heating to 100°C for 30 minutes. The number of experimental animals upon which the published values were based was not given. Shu Man Kyo published the information, in 1937 (43.), that a hormone of steroid nature existed in renal venous blood in rabbits. This hormone was able to lower the total lipid content in healthy-rabbit blood. It was also said to increase the lipase activity in peripheral blood.

The following is a report of my attempts to counteract hyperlipemia following bilateral ligation of the ureters, by means of injections of renal venous blood from healthy rabbits.

Bilateral ligation of the ureters was carried out on 11 rabbits (see chapter IV). Three of these received intravenously 10 to 15 ml. renal venous blood from healthy rabbits after the withdrawal of the same volume of blood. This was divided into two doses per 24 hours (see table 2).

The following operative technique was used in renal venous puncture. Pentothal was the anesthetic used. The abdomen was opened by means of a midline incision. The renal vein was exposed and compressed with a rubber-covered Péan forceps at the entrance to vena cava. The renal vein was then punctured with a fine needle. The Péan forceps was removed and the hole from the needle closed with a purse-string suture. The abdomen was closed.

As may be seen from the tables, no serum-lipid-decreasing effect whatsoever

could be demonstrated in renal venous blood from healthy rabbits. This was notwithstanding the "exchange" of as much as 40 percent of the total blood volume of the experimental animal.

Jiménez Diaz and Castro Mendoza, 1948 (44.), published a report of experiments with three dogs in which the hyperlipemia occurring after nephrectomy failed to appear if a glycerol extract from 1.4 Gm. pig kidney was administered daily. How this glycerol extract was prepared, or how it was injected, was not reported. The writers named the extracted lipid-metabolism-regulating factor nephrolipin. In 1949 (60.), the same writers described a similar effect on hyperlipemia following bilateral ligation of the ureters in dogs.

The following is a report of my attempts to affect the hyperlipemia which occurs after bilateral ligation of the ureters in rabbits by means of extract from healthy kidneys from rabbits, guinea pigs and pigs.

The extract was prepared as follows. The renal tissue was homogenized in physiologic saline solution or glycerol in the previously described homogenizer at 0° to +4°C. Thereafter, the tissue suspension was centrifuged one hour at 3000 revolutions per minute at +1°C. The extract was refrigerated between the injections.

The experiments were carried out on four rabbits with bilateral ligation of the ureters. The extract was administered immediately after the ligation was performed and subsequently twice a day. The extract in physiologic saline solution was injected half intravenously and half intramuscularly. The glycerol extracts were injected principally intramuscularly, inasmuch as only small quantities, diluted with physiologic saline solution, could be administered intravenously because of the tendency of glycerol to form emboli in the cerebral vessels. The doses appear in the table. The quantity of blood withdrawn for analysis was replaced with blood from healthy rabbits in the animals UR 8 and UR 14 (see table 2).

As is apparent from the table, the concentration of all lipids investigated in serum increased rapidly after ligation of the ureters despite administration of extract from normal kidneys. I did not consider it worth while to continue the experiments with extract prepared in the manner described. It is important, however, to point out once more that Jiménez Diaz et al. did not report how they prepared their glycerol extract. Therefore, it is impossible to duplicate their experiments in detail. It seems reasonable to believe, however, that the homogenization procedure used in this investigation should have great possibilities of extracting any glycerol-soluble hormones which might be present in the tissue. A further difference is the use of dogs by Jiménez Diaz et al. as experimental animals. Inasmuch as hyperlipemia after nephrectomy or ligation of the ureters seems to be a general occurrence in all animal species investigated, however, there is no conceivable reason to assume that the cause of hyperlipemia should not be the same in different mammals.

Miyazaki, Shu Man Kyo and Jiménez Diaz et al. interpreted the results of their investigations as meaning that a hormone is normally formed in the kidney which controls the lipid metabolism. Jiménez Diaz left the question open, however, as to whether there might also be a fat-splitting ferment, formed in the kidney and exercising its activity elsewhere.

Therefore, *an investigation of the activity of lipase (tributyrynase) in afferent and efferent blood from normal and "ureter-ligated" kidneys is also included in this work.* See table 6.

Table 6.

Tributyrynase activity in renal venous blood from normal and "ureter-ligated" kidney.

No.	Arterial	Venous	
NR ₅	4.1	4.1	Normal Kidney
NR ₆	4.2	4.2	Normal Kidney
UR ₉	4.3	4.0 4.6	Normal Kidney "Ureter-ligated" Kidney
UR ₁₀	3.6	4.2 4.6	Normal Kidney "Ureter-ligated" Kidney

Tributyrynase activity expressed in ml. 0,05 n-KOH.

NR = Normal (healthy) Rabbit. UR = "Ureter-ligated" Rabbit. Unilateral ligation of the ureter was performed on UR₉ and UR₁₀ >48 hours before the analyses were made. All enzyme activity determinations were made in serum.

It is apparent from the table that no positive difference could be established. (Reservation must be made for the viewpoints on such experiments discussed in greater detail in chapter VI.)

In summing up this experiment, it may be said that neither in renal venous blood nor in extract from normal kidney could any hormone be demonstrated which was able to inhibit the hyperlipemia occurring in rabbits after bilateral ligation of the ureters. Nor was any difference found in the lipase activity in the blood from afferent or efferent renal vessels of normal rabbits and those with ligation of the ureters.

Chapter VI

Attempts to Demonstrate Lipid Splitting in the Kidney by means of Differences between Lipid Concentrations in Arterial and Venous Renal Blood

Investigations reported earlier had not offered any explanation of the hyperlipemia which arises after nephrectomy or ligation of the ureters. I decided, therefore, to attempt to show *whether the kidney normally plays a role in the splitting of lipids and whether the hyperlipemia which occurs after nephrectomy or ligation of the ureters depends upon an interruption of the normal lipid-splitting function of the kidney.*

Achard et al. published the results of an investigation of the lipid concentration in afferent and efferent renal blood in 17 dogs, 1932 (61.). No detailed figures were reported. The writers reported finding a lower total lipid concentration (10 to 50 mg. per 100 ml.) in 13 dogs, a higher concentration (10 to 50 mg. per 100 ml.) in 3, and the same concentration in one. The venous cholesterol concentration was lower (5 to 15 mg. per 100 ml.) in 13 dogs, the same in four. Free fatty acid concentrations were lower (50 to 265 mg. per 100 ml.) in 12, higher (5 to 50 mg. per 100 ml.) in four and the same in one. The writers drew the conclusion from this that the dog kidney plays a role in the lipid metabolism of which we do not know the detailed nature. No appraisal of the results with respect to error in method was made. According to the reported figures, however, probably all differences shown fall within the limits of error of the method. Heymann and Clark, 1945 (57.), found no difference between lipid concentrations in renal venous blood, blood from the jugular vein and blood from the femoral artery.

General remarks on the foregoing experiment

The human kidney at rest is flushed by 2000 to 3000 liters of blood per 24 hours. If the total lipid concentration is determined in arterial and venous renal blood, for example, and such a small difference as 20 mg. per 100 ml. is shown (this is scarcely possible with the available methods even in a very large material) it would mean that the kidneys were able to break down 400 to 600 Gm. of lipids per 24 hours.

Furthermore, as pointed out in chapter III, the possibility of substrate accumulation and synthesis in the organ must be taken into account in such enzyme activity determinations. As far as the kidney is concerned, additional consideration

must be given to changes in concentration due to fluid loss in the passage of the blood through the kidney and to possible excretion of the substrate in the urine.

Substrate accumulation

Rabbits receiving vegetable diet have been used in the experiment described in this chapter. The lipid content in the kidneys did not increase despite rises in the serum lipid concentration, as stated in chapter IV. See table 5.

Lipid synthesis

Definite proof that the kidney is able to synthesize phospholipids was presented by Fisher, 1943 (62.). He found previously-administered radioactive phosphate in the same quantities in the phospholipids of the kidneys in dogs with hepatectomy as is found in similar experiments in the kidneys of dogs without hepatectomy. He found this notwithstanding that the plasma in the dogs with hepatectomy contained only traces of the labeled phospholipid.

Chaikoff et al. showed, 1950 (63.), that in vitro renal tissue could produce fatty acids from C^{14} -labeled glucose.

In 1950, Alfin-Slater et al. (64.) found previously-administered deuterium oxide in, inter alia, renal cholesterol. Chaikoff et al. showed, 1950 (65.), that when acetate labeled with C^{14} was administered to dogs which had undergone removal of the intestines and liver, C^{14} was found in renal cholesterol.

Excretion of lipids in the urine

Normal urine from man contains only traces of lipids. See Peters and van Slyke, 1946 (22.).

In order to prove that such was also the case in the animals used in this investigation, the lipid excretion per 24 hours was determined in nine animals and proved to be 10 to 20 mg. The animals had then been receiving standard diet. The urine was collected for several days.

Rise in lipid concentration in the blood due to fluid loss into the urine.

Because of the proportion between the quantity of blood passing through the kidneys (>2000 liters per 24 hours in man) and the daily volume of urine (1 to 2 liters), this increase in concentration is negligible.

It may therefore be considered probable that even if the kidney normally plays a large role in the lipid metabolism in the organism, the renal circulation is too rapid for the demonstration with the available methods of a possible slight fall in concentration in the passage through the kidney. Use of the method discussed is further complicated by the kidneys' taking part in the synthesis of lipids.

Determinations of the lipid concentrations in arterial blood (puncture of left chamber) and in venous blood from six normal and three "ureter-ligated" kidneys have been made.

Cholesterol analyses have been performed on whole blood in eight cases, inasmuch as, according to investigations by Bugnard, 1930 (66.), and Levine et al., 1939 (67.), inter alia, the cholesterol concentration in serum varies in arterial and venous blood in such a manner that cholesterol is shifted between serum and blood corpuscles. Six rabbits were used. Unilateral ligation of the ureters of three of them was carried out at least 48 hours earlier (i. e., a reduction of the triglycerol-

splitting capacity of the corresponding kidneys had been achieved, see chapter VII). The blood from both renal veins was analyzed in these three animals. See table 7.

Of course, a similar investigation can not be performed on man. In connection with seven kidney operations performed at Karolinska sjukhuset in the surgical department, the lipid concentrations in blood from arm and renal veins was studied. Consequently, no normal kidneys are included in this material but only

Table 7.

Lipid concentrations in arterial blood and in venous renal blood in rabbits.

No.	Total lipids			Cholesterol			Phospholipids		
	mg./100 ml.			mg./100 ml.			mg./100 ml.		
	Arterial	Venous	V-A	Arterial	Venous	V-A	Arterial	Venous	V-A
	d.v.	m.v.		d.v.	m.v.		d.v.	m.v.	
NR ₆	450	450	455	470	+20	71	70	71	+ 1
NR ₇	740	750	915	920	+170	42	42	45	47 + 5
NR ₈	895	900	865	880	—20	83	84	87	87 + 3
UR ₉	610	630	570	640	+10	89	90	98	98 + 8
UR ₁₀	900	920	1160	1160	+240	81	83	81	81 — 2
UR ₁₁	625	630	880	900	+270	88	88	100	101 +13
UR ₉	900	920	1030	1070	+150	81	83	88	90 + 7
UR ₁₁	625	630	714	710	+80	88	88	86	88 ± 0

All cholesterol concentration determinations except in animal NR₇ are made in whole blood.

All other determinations are made in serum.

V—A = Difference between concentration in venous and arterial blood.

n = Analyses made in blood from normal kidney.

u = Analyses made in blood from "ureter-ligated" kidney.

those in which nephrectomy or partial kidney resection was undertaken because the kidney was partially destroyed by disease. Furthermore, the comparisons were made, as stated, with venous blood from the arm and not, as was to be desired, with arterial blood. See table 8.

Table 8.

Lipid concentrations in serum from vena brachialis and vena renalis in man.

Name	Total lipids mg./100 ml.					Cholesterol mg./100 ml.					Phospholipids mg./100 ml.				
	Vena brachi- alis		Vena rena- lis		r-b	Vena brachi- alis		Vena rena- lis		r-b	Vena brachi- alis		Vena rena- lis		r-b
	d.v.	m.v.	d.v.	m.v.		d.v.	m.v.	d.v.	m.v.		d.v.	m.v.	d.v.	m.v.	
S.L.	980		900			233		200			6.9		6.8		
	960	970	940	920	-50	233	233	200	200	-33	7.1	7.0	6.7	6.7	-0.3
H.A.	545		530			186		203			5.4		5.0		
	605	580	570	550	-30	194	190	203	203	+13	6.4	5.8	5.5	5.2	-0.6
											5.5		5.0		
F.J.	440		583			169		160			4.1		5.9		
	580	510	500	540	+30	169	169	160	160	-9	6.1	5.0	7.5	6.9	+1.9
A.F.	1160		905			242		242			7.9		8.9		
	1090	1130	1000	950	-180	242	242	252	252	+10	8.2	8.2	9.2	9.2	+1.0
								261			8.4		9.5		
K.J.	707		780			112		155			4.7		5.8		
	733	720	825	800	+80	121	117	155	160	+43	5.2	5.0	5.8	5.8	+0.8
											5.0		5.8		
K.S.						264		215							
						264	264	229	222	-42					
E.J.						229		208							
						243	236	217	213	-23					

r-b = Difference between concentration in blood from vena renalis and vena brachialis.

As is apparent from the tables, the differences shown lay entirely within the limits of error of the method.

In summing up, it may accordingly be said that because of the rapid renal circulation, the synthesis of lipids in the kidney, and the uncertainty in the available methods of lipid determination, the role of the kidney in the breaking down of lipids can not be judged from concentration analyses in afferent and efferent renal blood.

Chapter VII

Determination of Lipase (Tributyrylase) and Phosphomono-esterase Activity in Kidney and Liver Suspensions and in Serum from Healthy Rabbits and from Rabbits on which Ligation of the Ureters Had Been Performed

In order to attempt in another manner to solve the problem as to whether the hyperlipemia which follows nephrectomy or ligation of the ureters depends upon an interruption of the normal lipid-metabolizing function of the kidney, the following experiment was carried out.

The lipase activity and the phosphomono-esterase activity at pH 4.8 and pH 10 were investigated in kidney and liver suspensions as well as in serum from healthy rabbits (NR = Normal [healthy] Rabbit) and from rabbits with unilateral or bilateral ligation of the ureters (UR = "Ureter-ligated" Rabbit). The lipid concentrations in the blood were studied at the same time in the manner described in chapter IV.

Homogenization of the tissue and determination of the enzyme activity was carried out as described in chapter III.

The results of the enzyme activity determinations in liver and kidney suspensions from healthy rabbits are presented in table 9.

As is apparent from the table, kidney tissue proved, on the whole, to have the same ability to split triglycerides as liver tissue had.

Enzyme activity determinations were made in the same way in liver and kidney suspensions from five rabbits with unilateral and six with bilateral ligation of the ureters. In those with unilateral ligation, the enzyme activity was determined in both kidneys. The results of the determinations from all "ureter-ligated" kidneys and from the liver in these animals are presented in table 10.

As shown by table 10, a considerable drop in lipase and alkaline phosphomono-esterase activity occurred in kidney tissue following ligation of the corresponding ureter. In liver tissue, on the other hand, no drop in activity could be shown. Acid phosphomono-esterase activity remained unchanged, on the whole, in both kidney and liver.

Table 11 shows the results of the enzyme activity determinations in tissue suspensions from both kidneys in five rabbits in which only one ureter was ligated. As may be seen, a pronounced decrease in lipase and alkaline phosphomono-esterase activity was found in the suspensions from the kidneys with ligated

ureters. The activity in the healthy kidney corresponded, on the whole, to the activity in normal animals, see table 9.

Experiments with corresponding quantities of tissue from healthy kidneys showed that with the quantities of tissue used for activity determinations, enzyme and substrate were present in proportions optimal to the activity. The drop in enzyme activity could not accordingly be explained by a decrease in the quantity of tissue used because of renal edema in the piece of tissue homogenized.

In the determination of the lipase activity in suspensions from healthy and "ureter-ligated" kidneys with reaction mixtures of different pH, it proved that after ligation of the ureters a congruent fall in the activity curve was obtained.

The decreases in enzyme activity in "ureter-ligated" kidneys was not affected by the administration of renal venous blood or glycerol extract from healthy kidneys (see chapter V).

The lipase activity in serum was determined in five rabbits before and more than 48 hours after bilateral ligation of the ureters (see table 12). *No drop in the lipase activity in serum after bilateral ligation of the ureters could be shown.**

In connection with nephrectomies or partial kidney resections performed in the surgical department of Karolinska sjukhuset, the lipase and phosphomono-esterase activity in tissue suspensions of microscopically healthy kidney tissue from nine patients was examined. The same examination was carried out on pieces of excised liver tissue from patients without sign of liver disease undergoing cholecystic operations.

It should be observed that this material comprises only kidneys from patients on whom surgery was undertaken because large parts of the kidney were destroyed by disease. Furthermore, it proved that various anesthetics had probably affected the enzyme activity. With reservation for these conditions, however, it may be said in summarizing these examinations, that they indicate that the proportions between the enzyme activities determined in the liver and kidney tissue from man agree on the whole with the corresponding enzyme activities in rabbits. More detailed results of these as yet incomplete studies will be published in connection with another investigation.

* According to R. Cerbone, 1941 (68.), a decrease in the lipase activity in skin, musculature and serum has been demonstrated following nephrectomy in dogs.

Therefore, the lipase activity was also determined in tissue suspensions from musculature in two rabbits. It proved to be so low that it could not be positively differentiated with this method from the lipase activity present in the blood to be found in the corresponding quantity of musculature.

Table 9.

Phosphomono-esterase and tributyrinase activity in tissue suspensions from healthy rabbits.

No. Weight in grams Sex	K i d n e y				L i v e r			
	Phosphomono-esterase		Tributyr- inase		Phosphomono-esterase		Tributyr- inase	
	Alkaline	Acid	Alkaline	Acid	Alkaline	Acid	Alkaline	Acid
	d.v.	m.v.	d.v.	m.v.	d.v.	m.v.	d.v.	m.v.
NR ₁ 3100 ♀	1.315	1.510	0.219	0.250	0.328	0.340	0.084	0.090
	1.697		0.273		0.356		0.097	
NR ₂ 2810 ♂	1.362	1.300	0.089	0.090				
	1.231		0.092					
NR ₃ 2480 ♂	1.213	1.230	0.270	0.260	528	480	0.833	0.153
	1.241		0.250		425		0.809	0.150
NR ₄ 2480 ♀	0.487	0.500	0.319	0.270	325	330	0.335	0.096
	0.505		0.222		337		0.391	0.183
NR ₅ 3200 ♀	0.916	0.780	0.234	0.240	493	470	0.103	0.151
	0.635		0.238		441		0.115	0.101
NR ₆ 2080 ♂	1.100	1.040	0.164	0.160	445	450	0.155	0.192
	0.972		0.164		445		0.169	0.181
NR ₇ 2240 ♂	0.792	0.800	0.213	0.180	487	520	0.124	0.117
	0.804		0.139		546		0.113	0.120
NR ₈ 2150 ♂	0.835	0.810	0.254	0.250	484	460	0.087	0.174
	0.792		0.255		440		0.074	0.147
Mean:	0.996		0.213		452		0.284	0.141
	+0.118		±0.022		± 26		±0.099	±0.012
								592
								± 38

Phosphomono-esterase activity is expressed in mmol liberated phenol per gram dry weight of tissue. Tributyrinase activity is expressed in ml. 0.05 N-KOH per gram dry weight of tissue.

Table 10.

Phosphomono-esterase and tributyrinase activity in tissue suspensions from "ureter-ligated" rabbits.

Sex	No. Weight in grams	Days after ligation	K i d n e y				L i v e r					
			Phosphomono-esterase		Acid		Phosphomono-esterase		Acid		Tributyr- inase	
			d.v.	m.v.	d.v.	m.v.	d.v.	m.v.	d.v.	m.v.	d.v.	m.v.
UR ₁ 2710 ♂		3	0.503		0.135				0.467		0.092	
				0.540		0.130			0.430		0.100	
			0.582		0.127				0.400		0.100	
UR ₂ 2070 ♂	x 4		0.324		0.213		277		0.756		0.059	507
				0.340		0.220	330		0.710		0.050	450
			0.353		0.221		385		0.669		0.046	395
UR ₃ 2190 ♂	3		0.605		0.193		276		0.828		0.078	615
				0.610		0.190	280		0.760		0.080	590
			0.618		0.187				0.694		0.083	571
UR ₄ 2460 ♂	2		0.633		0.205		125		0.253		0.197	641
				0.670		0.220	110		0.240		0.200	610
			0.697		0.242		86		0.224		0.210	580
UR ₅ 2530 ♂	4		0.225		0.462		172		0.203		0.203	558
				0.200		0.460	180		0.190		0.190	540
			0.185		0.461		192		0.170		0.176	516
UR ₆ 4120 ♀	x 23		0.089		0.179		13		0.201		0.062	408
				0.070		0.200	10		0.230		0.080	470
			0.042		0.211		13		0.266		0.106	539
UR ₇ 1900 ♂	3		0.648		0.250		247		0.184		0.166	507
				0.650		0.270	230		0.190		0.170	540
			0.658		0.286		203		0.198		0.170	576
UR ₈ 1940 ♀	2		0.267		0.305		181		0.179		0.165	670
				0.260		0.270	170		0.180		0.140	680
			0.250		0.237		151		0.187		0.120	680
UR ₉ 1890 ♂	x 6		0.386				179		0.432		0.123	754
				0.400	0.234	0.230	200		0.450		0.130	800
			0.419				227		0.461		0.141	838
UR ₁₀ 2530 ♂	x 4		0.423		0.352		328		0.144		0.081	568
				0.460		0.330	340		0.140		0.080	600
			0.490		0.310		360		0.135		0.081	631
UR ₁₁ 3170 ♂	x 10		0.110		0.342		88		0.084		0.174	464
				0.110		0.340	110		0.090		0.180	460
			0.104		0.344		125		0.091		0.182	449
Mean:				0.391		0.260	196		0.328		0.127	574
				±0.065		±0.027	± 33		±0.069		±0.016	± 34

x denotes unilateral ligation.
See table 9.

"ureter-ligated" kidney and from liver in rabbits with unilateral ligation of the ureter.

Normal Kidney				Liver					
Phosphomono-esterase		Tributyr-inase		Phosphomono-esterase		Tributyr-inase		Tributyr-inase	
Acid.				Alkaline		Acid.			
d.v.	m.v.	d.v.	m.v.	d.v.	m.v.	d.v.	m.v.	d.v.	m.v.
0.140		385		0.756		0.059		507	
	0.140		420		0.710		0.050		450
0.146		454		0.669		0.046		395	
0.201		398		0.201		0.062		408	
	0.190		320		0.230		0.080		470
0.179		238		0.266		0.106		539	
		390		0.432		0.123		754	
0.255	0.260		410		0.450		0.130		800
		439		0.461		0.141		838	
0.149		517		0.144				568	
	0.160		530		0.140		0.080		600
0.174		541		0.135				631	
0.254		291		0.084		0.174		464	
	0.250		260		0.090		0.180		460
0.247		231		0.091		0.182		449	

Discussion and Conclusions.

The hyperlipemia occurring in experimental animals following nephrectomy or ligation of the ureters in vegetable-fed rabbits may conceivably be caused by:

1. A decrease in the lipid-metabolizing capacity of the organism.
2. The occurrence of an increased lipid synthesis or mobilization of lipids from depots.

Ad 1: Examinations in this investigation have shown that:

- a. *Kidney tissue has the same triglyceride-splitting capacity as liver tissue, on the whole.*

As stated in chapter III, however, no direct conclusions may be drawn from the experiments concerning the enzyme activity of the organ in vivo with the enzyme activity determination methods used. Nevertheless, it seems justifiable to attempt to draw comparative conclusions concerning the roles of the liver and the kidneys in the metabolism of triglycerides. In doing this, it is necessary first of all to take into account that the quantity of tissue in the liver is greater than in the kidneys and that the volume of blood passing through the kidneys is greater than through the liver. These differences can be calculated mathematically. However, no definite conclusions can be drawn regarding the significance to the enzyme activity of the difference in circulatory volume. It seems probable that a rapid passage of blood with constant supplying of new substrate and removal of inactivating end products causes a rise in the enzyme activity of the organ. Numerical estimation of this rise is not possible, however.

Accordingly, it may be considered probable that the kidney plays a significant role in the break-down of lipids.

Investigations published earlier also lead to this conclusion. Quastel and Wheatley, 1933 (69.), showed by in vitro experiments using Warburg's method in an investigation of liver enzymes that kidney slices were also able to oxidize fatty acids. The published figures show that the same activity level was present in liver and kidney tissue. These results have subsequently been confirmed by Lehninger, 1946 (70.); Grafflin and Green, 1948 (71.); Geyer et al., 1949 (72.) and 1950 (73.); inter alia. Through the work of Hahn and Hevesy, 1939 (74.); Weissberger, 1940 (75.), and others, it is known that the kidney also takes part in the metabolism of phospholipids.

- b. *Following unilateral or bilateral ligation of the ureters, the lipase and alkaline*

phosphomono-esterase activity in the corresponding kidneys is sharply decreased.

The acid phosphomono-esterase activity, on the other hand, remains unchanged. *No corresponding decrease in the enzyme activity in the liver or serum can be shown.*

If the hyperlipemia which follows nephrectomy or ligation of the ureters depended upon a general toxic effect on cell enzymes, provoked by waste products retained in the blood, this decrease in the enzyme activity should be shown in the liver and blood also after bilateral ligation of the ureters. Furthermore, in the case of unilateral ligation it would not be possible to show any decrease in the enzyme activity of the "ureter-ligated" kidney.

That the acid phosphomono-esterase activity is not affected indicates that the drop in enzyme activity in the kidney after ligation of the ureter is not general.

This investigation has shown, accordingly, that a hyperlipemia occurs after bilateral ligation of the ureters and that a concurrent decrease in the lipase activity can be shown in the kidneys but not in the other organs. This seems to indicate, therefore, that *the hyperlipemia which follows nephrectomy, bilateral ligation of the ureters or ligation of renal vessels could be dependent upon an interruption or a decrease of the normal lipid-splitting function of the kidney.*

Ad 2: If the hyperlipemia following nephrectomy or ligation of the ureters depends upon an increased lipid synthesis or mobilization of lipids from depots, it must be provoked by either

a. the substances retained in the blood in the case of nephrectomy or ligation of the ureters. If such were the case, however, a more or less pronounced hyperlipemia would be a constant symptom of renal insufficiency. This does not appear to be the case.

A number of investigators who have attempted to correlate the hyperlipemia in cases of nephritis with a rise in nonprotein nitrogen have not been successful and have found that severe hyperlipemia can occur with a practically normal nonprotein nitrogen level. On the other hand, severe uremia is not always accompanied by hyperlipemia. "There is no inverse relation between the serum lipids and blood nonprotein nitrogen or any other index of renal excretory function" (Peters and van Slyke, 1946 (22.) page 531).

or b. the disappearance of a hormone which is normally produced in the kidney and has the ability to take part in lipid synthesis or mobilization of depot lipids. I have attempted, as described, to reproduce the results of earlier investigations which were said to indicate this, but have not succeeded.

It is therefore probable that the cause of hyperlipemia following nephrectomy or ligation of the ureters is not to be found in an increased lipid synthesis or mobilization of lipids from depots.

Hyperlipemia after nephrectomy seems to be a general phenomenon in all species of animals investigated; among them monkeys, in which it was especially striking. The fact that hyperlipemia has been described in man in cases of renal venous thrombosis further confirms the assumption that bilateral nephrectomy or ligation of the ureters would cause a hyperlipemia in man also.

It is particularly opportune, taking the results in this investigation as a basis, to discuss the question of the cause of the hyperlipemia which occurs in man in connection with nephritis and nephrosis.

Among earlier experimental investigations directly bearing upon this question, the work published in 1924 by Hiller, Linder, Lundsgaard and van Slyke (see review of literature) may be mentioned in particular. They reported an abnormal rise after a fat-rich meal in the free fatty acid and lecithin concentrations in the blood in patients with nephritis or nephrosis with hyperlipemia. In so far as it can be determined from the literature, this work has not been challenged. Page and Farr, 1936 (17.), studied the blood lipids in patients with nephritic or nephrotic hyperlipemia following the administration of a high or low fat diet. The lipid determinations were made in this instance, however, in a postabsorptive stage. No effect of the diet was observed. Both these investigations indicate that in these hyperlipemias there is a disturbed mechanism for the transfer of lipids from the blood to the tissue depots or a retardation of the break-down of the lipids.

As mentioned in the review of the literature, there are also many investigations in which the establishment of a correlation between lipemia and other symptoms in the disease picture in nephritis and nephrosis was attempted. Especially the question of a connection between edema and hyperlipemia has been the object of interest. Murphy, 1927 (76.), Maxwell, 1928 (77.), and 1934 (78.), Lichtenstein and Z. Epstein, 1931 (29.), believed they could demonstrate a connection. Similar experience was reported by Farr, 1942 (20.), in investigations with experimental nephrotoxic nephritis. The opposite opinion has taken precedence, however, in the reports of Calvin and Goldberg, 1931 (79.), Page, Kirk and van Slyke, 1936 (16.), and Peters and Man, 1943 (18.), inter alia. It is interesting in this connection to note that the most severe hyperlipemia has been observed in patients with pronounced edema. This has been particularly pointed out by Peters and van Slyke, 1946 (22.). That both these symptoms are pronounced does not necessarily mean, of course, other than that the disease is in a grave phase and that the hyperlipemia and edema are consequently concurrently increased. It should also be held in mind that the more detailed mechanism in cases of renal edema is not clear and that the degree of edema in the internal organs often eludes estimation in an external examination.

The cause of the decreases shown in this investigation in enzyme activities in the kidney following ligation of the ureter has not been discussed previously. It should be pointed out, however, that the dominating pathologic findings observed in a

"ureter-ligated" kidney are dilated canals and an extensive edema. It is impossible, of course, to draw conclusions from this as to the cause of decreases in enzyme activity.

It seems reasonable to assume that the lipase activity found in the kidney is principally in the cells of the tubules and not in the endothelium of the glomeruli. Accordingly, an explanation of the hyperlipemia in nephritis and nephrosis could be that hyperlipemia occurs in the phase of the disease when those cells of the tubules in which a strong lipase activity normally exists are affected.

Extensive but delimited pathologic renal processes may occur without concurrent signs of renal insufficiency. In the same way, judging from this investigation, large parts of the renal parenchyma can be destroyed without the occurrence of demonstrable deficiencies in the lipid-splitting function. To be sure, that is also true of the liver, of which 70 to 80 percent can be destroyed without demonstrable insufficiency. If the hyperlipemia in nephrosis and nephritis were of renal origin, it would consequently be provoked only by processes which affected the greater part of that renal parenchyma where lipase activity exists.

Many observations strongly favor the opinion that at least certain symptoms in the nephrotic syndrome indicate functional disturbances and not irreversible organic defects. A grave nephrotic condition with hyperlipemia can show sudden improvement so the patient is practically symptom-free after a relatively short time. There are numerous cases of nephrosis reported, for example, in which a dramatic recovery occurred in connection with infections. See reviews published by Blumberg and Cassady, 1947 (80.), Jiménez Diaz, 1950 (81.), *inter alia*.

In 1948, Barnet, Simmons and Wells (82.) described an instance of completely developed nephrotic syndrome in a patient with epilepsy, in connection with tridione therapy. The patient recovered after the tridione was discontinued, but suffered a relapse when the tridione was again administered.

White, 1949 (83.), described a similar case.

B. Olhagen and I have carried out experiments for the past two years, attempting to provoke nephrosis with tridione in animals. We have been able to produce hyperlipemia, proteinuria and fatty degeneration of the kidneys in rabbits, which resemble those conditions occurring in man in cases of nephrosis. But we have not been successful in producing the complete nephrotic syndrome in experimental animals.

These conditions argue strongly in favor of the hypothesis that at least certain symptoms in the nephrotic syndrome might depend upon reversible functional disturbances and not upon irreversible organic defects. *Disturbances in the renal enzymatic processes ought therefore to offer a reasonable explanation of the hyperlipemia in renal diseases.*

SUMMARY

The aim of the present investigation has been to study the cause of the hyperlipemia occurring after nephrectomy or bilateral ligation of the ureters. This hyperlipemia has earlier been found in rats, cats, dogs and monkeys.

Rabbits have been used as experimental animals in this investigation. A rapidly progressing hyperlipemia after bilateral ligation of the ureters was found in the rabbit also.

Administration of renal venous blood or tissue extract from normal kidney did not appear to affect this hyperlipemia. The writer has therefore been unable to verify the results of earlier investigations which were said to indicate that the kidney normally secreted a lipid-metabolism-regulating hormone.

The writer questioned then whether the cause of the hyperlipemia following ligation of the ureters could not be that the kidney normally takes part in the break-down of the lipids and that this function is reduced after ligation of the ureters.

The difficulties in the determination of the enzyme activity in living organs is discussed. The rapid passage of the blood through the kidney and the synthesis of lipids in the kidney prevent the determination of the function of the kidney in the break-down of lipids by means of lipid-concentration analyses in afferent and efferent renal blood. Analyses were made using rabbit and human blood.

An apparatus for the homogenization of tissue has been described. The comminution of the tissue cells obtained with this was such that a dispersed tissue suspension of cell fragments resulted.

The lipase (tributyrylase) and phosphomono-esterase activity was studied in serum and in kidney and liver tissue suspensions from healthy and "ureter-ligated" rabbits. Tissue suspensions from normal kidney showed the same triglyceride-splitting capacity, on the whole, as did liver tissue.

A considerable decrease in the lipase and alkaline phosphomono-esterase activity in kidney tissue occurred concurrently with a hyperlipemia in the animals with bilateral ligation of the ureters. No similar decrease in the enzyme activity could be shown, on the other hand, in serum and liver suspensions. In both kidney and liver suspensions, the acid phosphomono-esterase activity remained the same.

Lipase activities of the same order of magnitude were shown in the kidney and liver tissue in man.

The writer questions finally whether this investigation has not demonstrated

that the kidney normally plays an important role in the break-down of lipids and that the hyperlipemia which follows nephrectomy or bilateral ligation of the ureters is dependent upon an interruption of or a decrease in the normal lipid-splitting function of the kidney.

With these results as a basis, the writer also discusses the possibilities of determining the cause of the hyperlipemia occurring in connection with certain renal diseases.

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